THE UNIVERSITY OF ALBERTA

"The Behaviour of Some Substituted Anthraquinones at the Dropping Mercury Electrode"

A DISSERTATION

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DEPARTMENT OF CHEMISTRY

by

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ABSTRACT

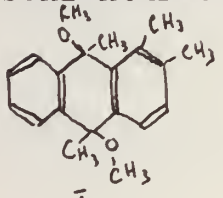
PART I

Various alkyl substituted anthraquinones were investigated at the dropping mercury electrode, and from the ease of reduction at the electrode, as indicated by their half-wave potentials an order of steric hinderance was derived:

1,2-dimethyl $>$ 1-methyl $\geq$ cyclohexano $>$ cyclopentano $>>$ H.

The effect of these substituents in the one position on the conjugation of the anthraquinone oxygens is discussed.

Considerable difficulty has been encountered in attempts to prepare the hydrocarbon 1,2,9,10-tetramethylanthraquinone. This compound is of interest as a model of the highly active carcinogen 9,10-dimethyl -1,2-benzanthracene.



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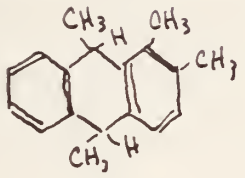

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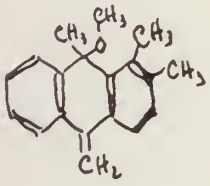

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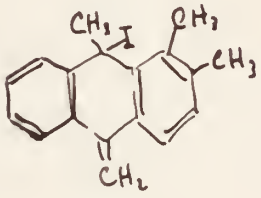


IV

Badger, Cook and Goulden (1) synthesized compound (I) from 1,2-dimethylanthraquinone, but found that the demethoxylation reaction of Bachmann and Chemerda (2) could not be affected even after shaking for six days with sodium powder in ether. In contrast to the behaviour of (I) 9,10-dimethylanthracene was obtained in good yield from the corresponding dimethoxy compound. Badger, Goulden and Warren (3) were unable to dehydrogenate compound (II) to the aromatic hydrocarbon. On the other hand 9,10 dimethyl- 9,10-dihydroanthracene was smoothly transformed into 9,10-dimethylanthracene when heated with sulfur at 230° C. The hydrocarbon 1,2,9,10-tetramethylanthracene has since been made by a new synthetic method (4), and became oxygenated after brief periods of storage in the solid state. More over there is some

experimental evidence in favour of the existence of derivatives which have the structure (III) and (IV). Because of these interesting facts which seem to depend upon the stability of the non-coplanar 9,10-dihydro derivatives of 1,2,-dimethylanthracene, it was decided to study the reduction of some alkyl substituted anthraquinones at the dropping mercury electrode. It was hoped that the polarographie half-wave potentials would show some dependence upon steric requirements of the carbonyl and alkyl groups.

The determination of the oxidation-reduction potentials was carried out by measuring the half-wave potentials of the anthraquinones at the dropping mercury electrode. The automatic recorder used was No.42200-A1. Electrochemograph manufactured by Leeds and Northrup. The cell was of the usual construction, viz. a dropping mercury electrode and a saturated calomel electrode as the positive and reference electrode.

Three buffer solutions were tried, Table I, these buffers acted as indifferent electrolytes as well as controlling the pH, which can greatly affect the value of the half-wave potentials as is shown in Table III. The value $E_{\frac{1}{2}}^{\circ}$ was determined by use of the equation

$$E' = E_{\frac{1}{2}} - 0.0591 \log [H^{+}]$$

where the $[H^{+}]$ indicated is that of the quinone solution used in determining the half-wave potentials.

A small amount of quinone, whose half-wave potential was to be measured, was placed in the alcoholic solution, warmed, and the temperature was allowed to drop to 25 C. The resulting saturated solution was filtered, to get rid of the excess quinone, and placed in a constant temperature bath at 25.0±0.1° C. Nitrogen gas, which had first been passed through a 0.5M solution of chromous chloride, a 1M solution of sodium hydroxide, a 0.1M solution of mercuric chloride, and the stock solution used to dissolve the quinones, was then passed through the saturated solution for twenty-five minutes. The height of the mercury reservoir was adjusted to allow a drop time of one drop for every four seconds from the dropping mercury electrode. No maxima were encountered in any of the

TABLE I

BUFFER - INDIFFERENT ELECTROLYTE SOLUTIONS USED.

No.	Buffer		Solvent	pH
1.	$(CH_3)_4NOH$	0.2N	50% Isopropyl Alcohol	12.0
2.	$NaC_2H_3O_2/HC_2H_3O_2$	0.1N	50% Isopropyl Alcohol	5.6
3.	$NaC_2H_2ClO_2/HC_2H_2ClO_2$	0.1N	50% Isopropyl Alcohol	4.5

The solutions referred to in Table I are all aqueous.

TABLE II

HALF-WAVE POTENTIALS OF VARIOUS SUBSTITUTED ANTHRAQUINONES.

Carried out 25.0 ± 0.1°C. and at pH 12.0

Quinone	$-E_{\frac{1}{2}}$ vs. Saturated Calomel Electrode	E°
anthraquinone	0.81 V	0.15 V
1-methylanthraquinone	0.95	0.01
1'2'3'4'-tetrahydro-1,2-benzanthraquinone	0.94	0.02
1,2-cyclopentanoanthraquinone	0.89	0.07
1,2-dimethylanthraquinone	0.99	-0.03
1,4-dimethylanthraquinone	1.04	-0.08

TABLE III

Buffer	pH	$-E_{\frac{1}{2}}$ vs. S.C.E.[i]	$+E^{\circ}$	
$0.2N(CH_3)_4NOH$	12.0	0.81 V	~~0.35~~ V	+0.15
$NaC_2H_3O_2/HC_2H_3O_2$	5.6	0.56	~~0.48~~	+0.02
$NaC_2H_2Cl\ O_2/HC_2H_2Cl\ O_2$	4.5	0.45	~~0.43~~	+0.07

i. S.C.E. - Saturated Calomel Electrode

The anthraquinones used were previously prepared by S. Levine and freshly recrystallized from ethyl alcohol. The isopropyl alcohol was Nichol's Chemical Company 98% reagent grade. Tetramethyl ammonium hydroxide was that manufactured by Eastman Kodak Co. Ltd. and marketed as the ten per cent aqueous solution.

It is apparent from the equation

$$E^{\circ} = E_{1/2} + 0.0591\ pH$$

that for two quinones the differences in their half-wave potentials are the same as the differences in their E° potentials, provided they are measured at the same pH and in the same buffer solution. Furman and Stone (5) suggest that the irregularity in the values of E° in various buffer solutions is due to the complexing of the quinone with the buffers, table III.

It should be noted that the value for $E_{1/2}$ for anthraquinone compares favourably with those given by Wawozonek, Laitinen and Kwiatkowski (6) which is -0.76V vs. S.C.E., and in the case of Furman and Stone (5) -0.79V vs. S.C.E.at pH 11.2 The discrepancy may arise in choice of solvent as both authors used 40% dioxane and an indifferent electrolyte - buffer system other than that used in obtaining the results of table II.

The steric affect of the alkyl group upon the ability of the quinones to reduce at the dropping mercury electrode appears to have a definite order, i.e.

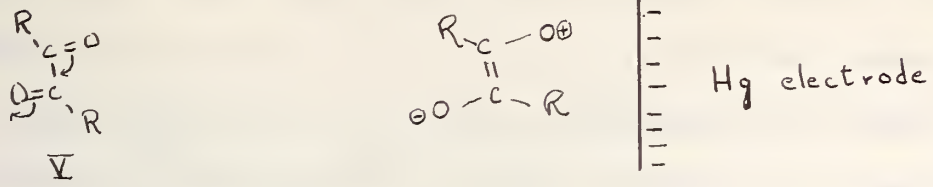
1,2-dimethyl>1-methyl>>tetrahydrobenz>cyclopentano>>H

This order is in agreement with that of Arnold and Craig (7) obtained from ultra-violet absorption studies, as well as that of Golumbic and Orchin (8) from partition studies of phenols. The results do vary, as do the results of the other workers, from that of Kadesch (9) who states the order of steric hinderance as

methyl>7 member ring>5 membered ring>6 membered ring

Leonard, Laitinen and Mottus (10) showed that in the dicarbonyl system (V) the more bulky the group

R the more resistant the system is to reduction, i.e.



V

the more negative the half-wave potential. In the case of the diketone system described by Leonard the sheltering affect is due to the inability of the carbonyl group to approach close to the dropping mercury electrode (VI). This was shown by the fact that in changing R from methyl to tertiary butyl a decrease of 0.46 V was incurred. Leonard states that both carbonyl groups are involved in such a reduction and that the reduction depends upon the steric environment of the dicarbonyl system to become coplanar. The resonance between the two systems being greatest when the two carbonyl groups are coplanar, i.e. 180° or 0° to one another, and least when perpendicular to one another (10).

In the case of anthraquinone the two carbonyl groups are coplanar and under the influence of the electrode would be polarized and reduced as in Figure 1. Thus the movement of the electrons from the electrode to the polarized

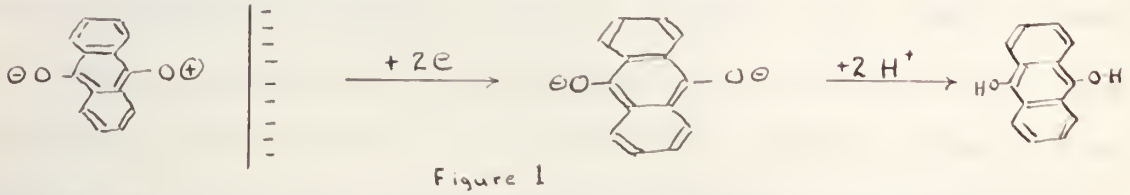
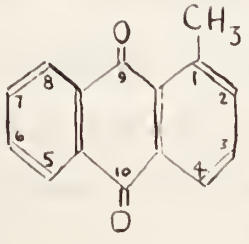


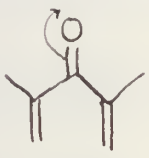
Figure 1

molecule is not impaired and the reduction is relatively easily carried out. If a methyl group is placed in the one position we find a decrease in the oxidation - reduction

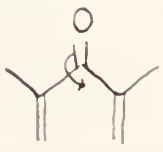


potential, i.e., from -0.81V to -0.95V. This at first would 8 appear to be anomolous in as much as the ten carbonyl oxygen is still free from any steric hinderance, and should be capable of polarization in such a manner as to carry out a non-hindered reduction at the ten carbonyl group, just as in figure 1. On closer inspection of a Fischer-Herschfelder-Taylor model we see that there is a steric strain between the one methyl and the nine carbonyl's oxygen. This strain could be alleviated by the oxygen taking up a position out of the plane of the rings.

Such a polarization of a carbonyl double bond could come about only by the formation of an oxonium ion and a carbanion. The polarization in the normal manner (VII) ascribed to a carbonyl would yield a carbonium ion which is uniplanar thus the oxygen would still be in the plane of the



VII



VIII

ring. (11) But by polarizing the group as in (VIII) we have produced a carbanion and an oxonium ion. The influence of such a sterically induced polarization would affect the ten carbonyl group in such a way that it could not polarize with the ease possible in the non-hindered anthraquinone. If it did, the aromatic sextet would be regenerated and would result in sp^2 bond hydridization restoring the -O- to the plane of the ring. The polarization of the ten carbonyl double bond in the manner illustrated in (VIII) would be difficult as we already have a high electron density in the ring, due to the sterically induced polarization of the nine carbonyl.

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ABSTRACT

PART II

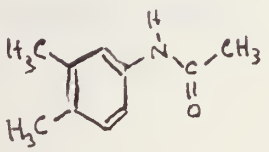
A.

The synthesis of 3,4-dimethylacetanilide by three methods in outlined in order to try and discern as to why the material has proved carcinogenic and non-carcinogenic at various times.

B.

The synthesis of 3-hydroxy-4-acetaminobiphenyl has been carried out. The material was made as it is a suspected metabolite of the carcinogen 4-acetaminobiphenyl, and the possibility that the metabolite was a carcinogen required investigation. It proved to be non carcinogenic.

On first examination 3,4-dimethylacetanilide (I), as made by the procedure of Zaugg (1), was believed to be an active carcinogen. The material was tested as an analogous compound to the known carcinogen 2-acetaminonaphthalene (II) (2), on the basis of the hypothesis that the o-dimethyl arrangement is equivalent to a fused ring.



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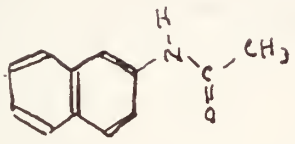

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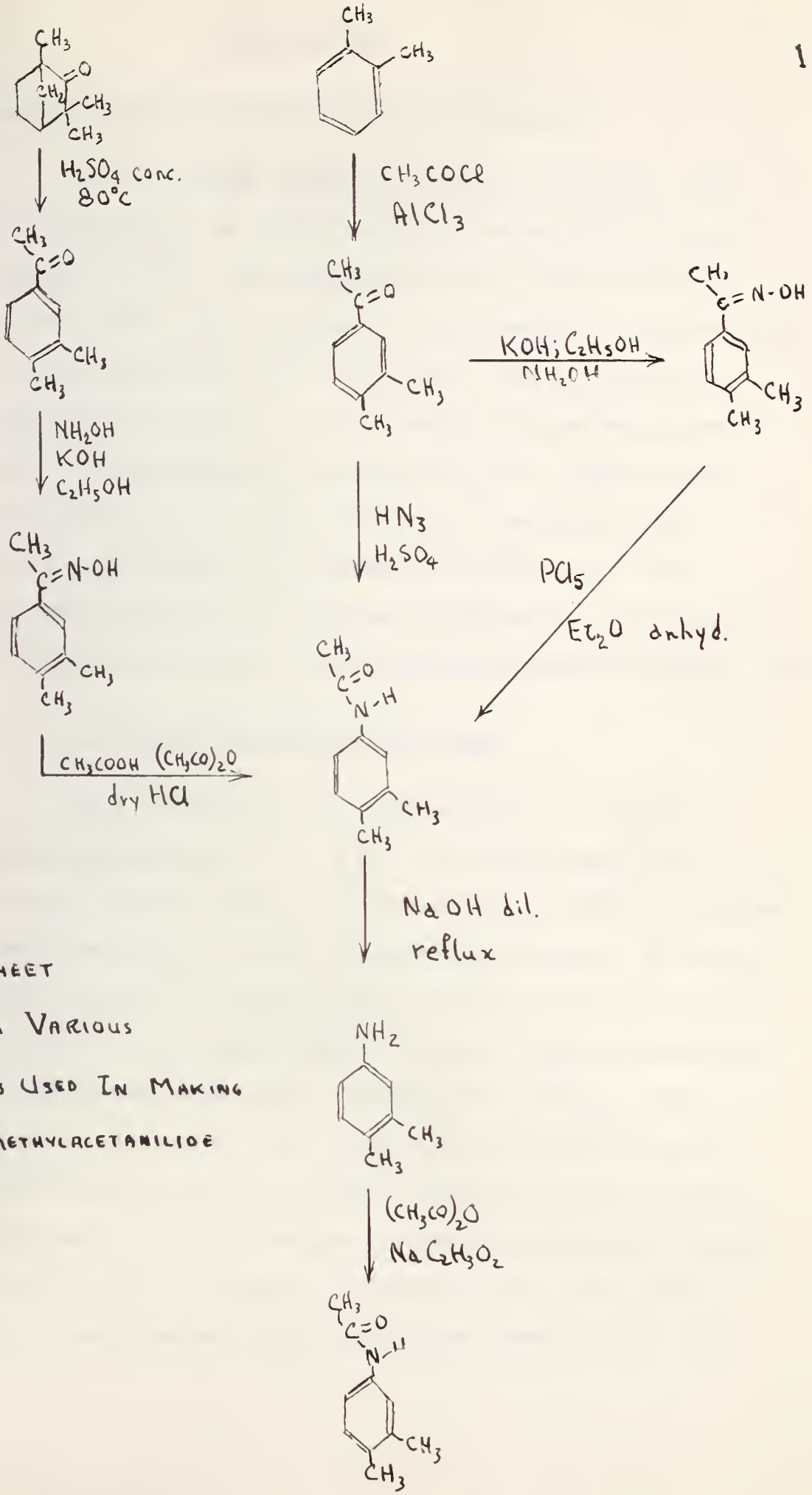
The procedure first used involved the rearrangement of fenchone in concentrated sulfuric acid, followed by oxime formation of the resulting ketone. The oxime was then put through the Beckmann rearrangement and the resulting xylidene acetylated and purified. This material upon feeding to rats proved carcinogenic.

At a later date more material was desired for further investigation of its carcinogenic nature. A different procedure giving increased yields with less difficulty was used. But, this second material proved to be non-carcinogenic. The question arose as to whether the second material contained an inhibitor, or did the initial material contain a carcinogen as a contaminant?

In order to ascertain the true nature of 3,4-dimethylacetanilide, it's synthesis was carried out by yet another method. This was done under the assumption that if the material by the third method proved to be carcinogenic, then that made by the second procedure carried an inhibitor as an impurity, possibly an azide. If the material by the third method did not prove carcinogenic then it was assumed that the original material contained an impurity which gave it it's carcinogenic nature.



FLOW SHEET

SHOWING VARIOUS

METHODS USED IN MAKING

3,4- DIMETHYLACETANILIDE

3,4-Dimethylacetophenone from Fenchone (1)

Four hundred grams of fenchone was added, with vigorous stirring, to 1600 ml. concentrated sulfuric acid and heated to 80°C. The temperature was not allowed to rise above 110°C. and was kept in this range for ten minutes after the completion of the addition. The hot solution was then poured into 6 l. of ice water and extracted with benzene. Distillation of benzene from the organic layer of the steam-distillate gave 500 g. of residual deep yellow oil containing 3,4-dimethylacetophenone. The product was purified by a vacuum distillation. Yield 200 g. about 50% of theoretical, boiling point 138-43°C. (55 mm. Hg.).

3,4-Dimethylacetophenone Oxime

To a solution of the crude yellow oil of 3,4-dimethylacetophenone in 1.5 l of 95% ethyl alcohol was added with stirring 450 g. of hydroxylamine sulfate followed by a cold solution of 608 g. potassium hydroxide in 1600 ml. 50% ethyl alcohol. Stirring was continued without external heating for 24 hours and then 520 ml. of concentrated hydrochloric acid was added with stirring and cooling. The precipitated inorganic salts were filtered with suction and the filtrate was boiled with norite for 10-15 minutes, and filtered. The temperature of the filtrate was adjusted to 50-55°C. and just enough 95% ethyl alcohol was added to make a homogeneous solution at this temperature. Any

inorganic salt precipitate at this point was filtered off, the filtrate (50-55°C) was seeded with some oxime and allowed to stand overnight at room temperature. Refrigeration for several hours followed by filtration gave 300 grams of crystalline 3,4-dimethylacetophenone oxime (M.P. 82-85°C.).

When 3,4-dimethylacetophenone of a higher degree of purity was used, such as obtained by the acetylation of o-xylene, the 3,4-dimethylacetophenone oxime came down with the inorganic salts and was obtained by merely washing the material on the Buchner funnel with a large amount of water.

Beckmann Rearrangement of 3,4-Dimethylacetophenone Oxime (1)

A solution of 300 g. of the oxime in 720 ml. glacial acetic acid and 455 ml. of acetic anhydride was cooled in ice and saturated with gaseous hydrogen chloride. The solution was then allowed to stand at 40°C. for 60 to 70 hours, cooled in ice and filtered, (using a sintered glass funnel) with suction, and as much solvent as was possible was pressed out of the filter cake.

The filter cake was immediately mixed with 550 ml. concentrated hydrochloric acid and refluxed until a homogeneous solution was obtained (approximately two hours). The 3,4-dimethylaniline hydrochloride could be obtained merely by crystallization of cooled reaction mixture. However to obtain the pure base, the reaction mixture

was made basic with sodium hydroxide, extracted with ether, dried and distilled. In this manner there was obtained 106 grams of pure 3,4-dimethylaniline (b.p. 132-4°C./45 mm. Hg) (m.p. 50-51°C.).

The preparation of the acetyl derivative was carried out by the procedure of Fieser (3).

3,4-Dimethylacetophenone from O-Xylene

One hundred fifty grams of anhydrous aluminum chloride was placed along with a solution of 106 g. o-xylene in 500 ml. carbon disulfide in a 2 l. three necked flask fitted with a stirrer, dropping funnel, and Tamworth condenser. The acetyl chloride, 78 g. was added dropwise to the mixture. The mixture was stirred constantly, and refluxed until the evolution of hydrogen chloride ceased. The material was decomposed by pouring into ice water, followed by heating on a steam bath until all the carbon disulfide was evaporated off. An ether extraction was then carried out on the resulting mixture, followed by drying over anhydrous calcium chloride. The ether was then distilled off and the 3,4-dimethylacetophenone purified by vacuum distillation. (b.p. 125°C. at 20 mm. Hg). Yield 122 g. 82% of theoretical.

Reaction of Hydrazoic Acid on 3,4-Dimethylacetophenone (4)

To a solution of 21.4 grams of 3,4-dimethylacetophenone was added, with stirring, 30 ml. of concentrated sulfuric acid. One hundred twenty three ml. of a 6.4%

hydrazoic acid solution in benzene was added dropwise with stirring. The temperature was maintained at 40°C. during the addition, which took approximately 50 mins., then cooled and poured into a separatory funnel. The concentrated sulfuric acid layer was poured into 400 ml. of ice water, and the solution made alkaline with ammonium hydroxide (120 ml.). The precipitate was filtered off, washed once with water and refluxed for two hours with concentrated hydrochloric acid. The solution was poured into 250 ml. of water, made alkaline, and extracted with diethyl ether. The ether layer was then dried overnight over anhydrous sodium sulfate. Distillation of the amine under reduced pressure after removal of the ether gave a colorless liquid (b.p. 120°C. at 18 mm. Hg.). The yield of 3,4-dimethylaniline was 14.5 grams, 85% of the theoretical.

Beckmann Rearrangement of 3,4-Dimethylacetophenone Oxime Using Phosphorus Pentachloride

One hundred grams of the oxime was dissolved in 300 ml. of anhydrous ether in a 2 l. Erlenmeyer flask fitted with an Allhyn condenser. The phosphorus pentachloride (150 grams) was added carefully over a period of 20 minutes. After the evolution of heat subsided the mixture was refluxed for an additional 30 minutes. The phosphorus pentachloride was decomposed by adding water dropwise through the condenser. The ether layer was distilled off and the resulting aqueous layer refluxed for one hour until the hydrolysis of the amide was complete. The amine was then purified by the same method as described previously for 3,4-dimethylaniline.

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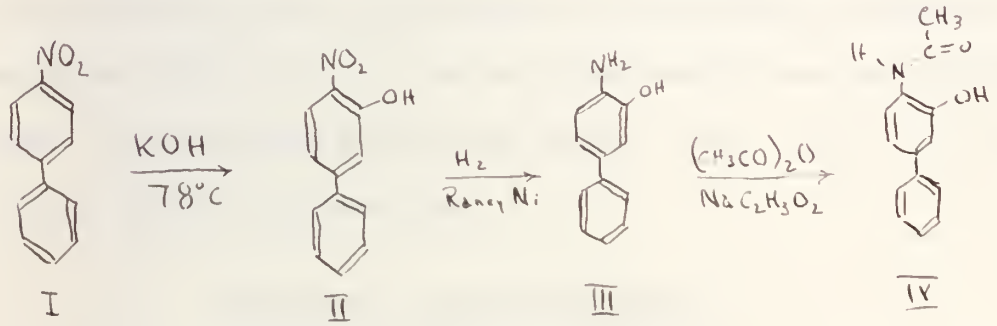
Bonser, Clayson and Dull (1), in an investigation as to the possible cause of bladder tumours found in people engaged in the aniline dye industry, were able to establish the fact that the aminonaphthalenes and some of the hydroxy-aminonaphthalenes are carcinogens in experimental animals. As a matter of course 1-amino-2-hydroxynaphtalene was investigated, and found to be as active a carcinogen as 3-methylcholanthrene.

Biologically hydroxylated products of carcinogens have been found to exist in the excreta of rabbits, mice and rats by Boyland and Levi (2) and Chalmers and Peacock (3). Berenblum and Schoental (4) found that the metabolite of 1,2-benzanthracene, a strong carcinogen, is 4-hydroxy-1,2-benzanthracene. Bielshowsky (5) has shown that rats recieving 2-acetylaminofluorene excrete 2-acetylamino-1-hydroxyfluorene in the urine. It was thus deemed desireable to investigate 3-hydroxy-4-acetylaminobiphenyl for its carcinogenic properties as it is a likely metabolite of the known carcinogen 4-acetylaminobiphenyl.

The fact that 2-amino-1-hydroxynaphthalene and 1-amino-2-hydroxynaphthalene were both found to be active carcinogens made the study of 3-hydroxy-4-acetylaminobiphenyl, and 3-hydroxy-4-aminobiphenyl desireable from the standpoint of comparing the carcinogenic nature of the fused ring system of naphthalene with that of biphenyl.

Various routes were tried in the synthesis of 3-hydroxy-4-aminobiphenyl (III), such as the reduction of 3-nitro-4-acetylaminobiphenyl followed by diazotization and hydrolysis of the diazonium salt to yield the phenol. This was found unsuccessful as triazole formation resulted. The diazotization of 3-amino-4-nitrobiphenyl was also attempted but the desired product could not obtained from the resulting tar. The nucleophilic attack of the hydroxyl ion on the nitro compound was first carried out by Colbert, Meigs and Jenkins (6) who reported at 14-16% yield. By a different method of purification the yield was increased to 50%.



I II III IV

3-Hydroxy-4-Nitrobiphenyl (6)

Five hundred grams of finely ground potassium hydroxide and 200 g. of powdered 4-nitrobiphenyl in 200 ml. benzene were heated at 78°C. for a total of 72 hours. A small amount of benzene and water was added and the resulting mixture filtered by suction. The pulp material was then acidified in aqueous media and collected by suction filtration. The crude reaction product was then dissolved in benzene and extraction with 10% sodium hydroxide carried out, until upon acidification of the alkaline layer a precipitate was no longer formed. The alkaline layer was acidified and the 3-hydroxy-4-nitrobiphenyl collected by suction filtration. The crude material was recrystallized from high-boiling petroleum ether, (m.p. 102-3°C.) yield 110 g. 50% of theoretical.

4-Amino-3-Hydroxybiphenyl

Ten grams of 3-hydroxy-4-nitro-biphenyl was dissolved, with heating, in 50 ml. ethyl alcohol and 50 ml. of ethyl acetate. Raney nickel catalyst was added and the nitro compound reduced in a Parr Low-Pressure Hydrogenation apparatus. After two hours the reduction was complete and the Raney nickel was filtered off. The mixture was boiled to half volume and an equal amount of water was added. The amino-phenol then crystallized out on cooling. The product was filtered and recrystallized from 60% ethyl alcohol. Yield 7.3 g. (m.p. 185-6°C. darkening at 180°), 85% of theoretical.

Analysis	Calculated for $C_{13}H_{11}NO$	C 78.80% ; H 6.03%
	Found	C 78.21% ; H 5.98%

3-Hydroxy-4-Acetylaminobiphenyl

The amine was acetylated using the procedure of Fieser (7).

The product isolated was soluble in 5% sodium hydroxide but insoluble in 5% hydrochloric acid. It was recrystallized from 70% ethyl alcohol (m.p. 194-5 C) Yield 90% of theoretical.

Analysis	Calculated for $C_{14}H_{12}NO_2$	C 74.00% ; H 5.69%
	Found	C 73.63% ; H 5.74%

The hydroxylated acetylaminobiphenyl did not prove carcinogenic in the animals under examination (8). This would mean that the fused ring of naphthalene and the biphenyl rings are not equivalent as carcinogens, possibly due to a difference in planarity, and electron densities in the rings.

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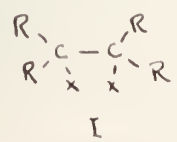
ABSTRACT

PART III

Certain tetrakis-(p-dimethylaminophenyl)-ethylene addition products were investigated for cytological activity and encouraging results attained.

The mode of action of nitrogen mustard as a drug in the treatment of leukemia is not yet fully understood. However there appears to be several necessities from the stand point of functional groups in the molecule (1). The formation of a carbonium ion by the removal of a halogen in polar solvents is a property attributed to most of the compounds having the desired cytological activity. This removal may be facilitated by a desirable inductive effect of certain groups in the molecule. We know that alpha-halo compounds, such as benzyl chloride, are capable of readily forming a carbonium ion due to the resonance stabilization of the ring. This property is enhanced by having para to the benzyl group another group having -E activity, that is an electron donor (2).

It also appears necessary in order that a molecule possess cytological activity that it should be bifunctional, that is have the property of carbonium ion formation at at least two points in the molecule. This may be effective in the molecule having the ability to cross-link the functional groups of protein (3). These factors along with a general interest in compounds possibly having a halonium ion in their structure led to the synthesis and biological investigation of compounds having the general structure (I).



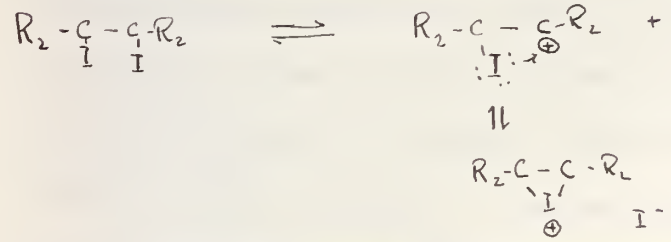
I

R = p-$C_6H_5N(CH_3)_2$

X = I, Br, Cl

In the case where the substituents R are p-dimethylaminophenyl the double bond of the corresponding ethylene is readily added to by halogens. The formation of periodides

by the iodine addition products would tend to subscribe to the belief that the halogens exist in an ionic manner in the molecule (4). There also exists the possibility of Halonium ion formation.



This would mean the isolation of a three-membered halonium ring (5), which has been postulated to exist in solution, but never isolated. This belief is augmented by the fact that A. Hay (6) in measuring the degree of dissociation of the dichloro compound by the freezing point depression method found that a tendency towards the value n 2 was encountered in dilute solutions.

Philips, Biescle et al (8) believe that cytotoxic action is dependent upon the presence in the compound, or its active intermediate, of an unstable three-membered heterocyclic ring system.

The halonium salts have previously been found very effective "in vitro" against tuberculosis bacillus (8), but its toxicity proved to great "in vivo" to be effective.

Preparation of tetrakis-(p-dimethylaminophenyl)-ethylene

Michler's ketone 5 g. was dissolved in 75 ml. concentrated hydrochloric acid along with 9 g. of tin foil. The solution was allowed to stand for 2 hours and refluxed for an additional 2 hours. On cooling the yellow double salt of stannic chloride crystallized out. This salt was collected by suction filtration , and dissolved in water followed by the addition of sodium hydroxide until the solution turned red litmus to blue. The material was then extracted with warm chloroform, followed by concentration and cooling of the chloroform extract. The material which separated out was filtered, dried, and recrystallized from 95% ethyl alcohol. Yield 2.5 g. 55% theor. (M.P. 297-303° C.).

Preparation of 1,2-dichloro-1,1,2,2-tetrakis-(p.dimethamino-phenyl)-ethane (10)

A solution containing 1.75 g. of the ethylene hydrocarbon dissolved in 150 ml. CCl_4 was shaken continuously as chlorine gas was passed over. When a brown decomposition material began to form at the top the chlorination was stopped. The solution was allowed to sit hour before filtering and the resulting dichloride was purified by dissolving 1.5 g. in 100 ml. chloroform followed by the addition of 150 ml. carbon tetrachloride. The product dissolves in water producing a blue solution.

Preparation of 1-chloro-2-iodo-1,1,2,2-tetrakis-(p-dimethyl aminophenyl)-ethane

This preparation was carried out in exactly the same manner as the iodo-chloro compound except that a solution of iodine in carbon tetrachloride was used. The compound yielded was dark green in colour and melted with decomposition at 94-97° C.

DISCUSSION

The influence of the halogenated tetraphenyl ethanes upon tumorus was investigated by injecting rats subcutaneously twice weekly with doses of 1.2 g./Kg: of body weight, and 2.4 mg./ kg. of body weight. The rats were fed the carcinogen p-dimethylaminazobenzene (DAB) and a group were held as a control. For comparative purposes another group also fed the DAB were injected semi-weekly with 1 mg./kg. of body weight of nitrogen mustard.

Of the halogenated materials only the dichloro-tetrakis-(p-dimethylaminophenyl) ethane (TDE) was examined and the effect of the material on the leukocytes was determined forty hours after subcutaneous injection of the TDE and nitrogen mustard. (TABLE 1).

The TDE shows the properties of a radiomimetric drug, so far as examined. The leukocyte count is decreased and the bone marrow is affected. Further investigation revealed an ability to prevent tumour formation in rats fed DAB up to 120 days. During the course of the experiment there was a noted difference in the appetite of the animals, those injected with TDE eating healthily, whereas those fed nitrogen mustard were the smallest eaters.

Two of the rats on nitrogen mustard died after ten weeks of injections, whereas those on TDE were not notably affected over the control rats. It is known that

nitrogen mustard sets up a state of shock which is reflected by an aleukemic condition. This was not evident on those rats injected with TDE.

TABLE I

LEUKOCYTE COUNT 40 HOURS AFTER SUBCUTANEOUS INJECTION of TDE and N-MUSTARD in RATS

Treatment	No. of Rats	Leukocyte	Average
Control	15	8,300 - 13,500	10,000
1.2 mg./Kg T.D.E.	5	4,500 - 6,450	5,620
2.4 mg./Kg T.D.E.	5	3,750 - 5,000	4,178
N Mustard 1 mg./Kg	5	1,150 - 1380	1,380

The effect of TDE on the bone marrow was considered to be different from that of Nitrogen Mustard, and is as yet under inspection.

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